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Serotyping and Genotyping of Hepatitis C Virus (HCV) Strains in Chronic HCV Infection

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Hepatitis C virus (HCV) genotypes can be established by methods based on PCR typing and serological typing. The accuracy of these methods depends on their sensitivity and specificity. These should be compared with the reference method, direct sequencing, and analysis of viral genomes. Among the serologic methods recently developed, the performance of a new serotyping assay (RIBA HCV 3.0 SIA, Chiron corporation, Emeryville) was assessed using a panel of 147 well-characterized French isolates from chronic hepatitis C patients. Definitive genotypes of the isolates were established by direct sequencing in 5′ NC and in some cases in NS-5B. HCV serotypes 1, 2, and 3 were determined by measuring type specific antibodies to core and NS-4 derived peptide antigens. Of the 147 sera, serotypic-specific antibodies were detected in 136 (sensitivity, 92.5%). The specificity of the RIBA SIA HCV serotyping assay was 92.6% (including samples with mixed results); without these, the specificity was 80.1%. Analysis of the 28 discrepant samples showed that (1) a different serotype was found in 18 samples including five for genotype 1, three for genotype 2, two for genotype 3, five for genotype 4, and three for genotype 5, and that (2) ten patients showed a reactivity with mixed serotypes, one had circulating antibodies to type 1 or 2, and nine had circulating antibodies to type 1 or 3. In summary, except for genotypes 4 and 5, the results of the test were well correlated (85.7%) with those of direct sequence genotyping. The former test is rapid and does not require the strict HCV RNA storage and preservation conditions of the latter. This new method may thus be considered as an alternative for HCV typing. However, although it is convenient, its lower sensitivity compared to the molecular typing method and the discrepant results limit its routine use in a clinical context. ***J. Med. Virol. 52:391–395, 1997.***

KEY WORDS: HCV Serotypes; HCV genotypes; HCV sequence analysis; HCV typing

INTRODUCTION

Hepatitis C virus is considered the major etiologic agent of post-transfusion non-A, non-B hepatitis. The viral genome, a positive single strand RNA of about 9400 nucleotides [Choo et al., 1989], is characterized by a high genetic heterogeneity, as are other RNA viruses. Four levels of genetic variability have been described: types, subtypes, isolates, and quasispecies. The nucleotide sequence data on the different isolates in the NS-5 [Holmes et al., 1993] core [Bukh et al., 1994], E1 [Buck et al., 1993] of NS-4 region [Bhattacherjee et al., 1995] served to classify HCV into six major genotypes, designated HCV-1 to HCV-6 [Simmonds et al., 1993a]. Further sequence analysis of HCV isolates from Vietnam revealed the presence of additional major genetic groups designated HCV-7 to HCV-9 [Tokita et al., 1994]. However, some research groups have suggested that these HCV types are more extreme variants of HCV-6 [Simmonds et al., 1995]. A relationship exists between particular types and the geographical source of infection in European patients [Pawlotsky et al., 1995]. Also, the response to interferon alpha therapy and the clinical outcome of the disease may be influenced by the virus type [Martinot et al., 1995; Nousbaum et al., 1995; Tsubota et al., 1994].

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Several methods have been described for typing hepatitis C virus. These have been generally compared with direct sequence analysis, considered the reference method. Two categories of methods are used currently. The genotyping methods are based on the analysis of virus RNA by RT-PCR. These methods are able to detect types and subtypes and are separated into type-specific PCR primers [Okamoto et al., 1992; Chayama et al., 1993], restriction fragment length polymorphism [Simmonds et al., 1993b], and reverse hybridization [Stuyver et al., 1993].

Serologic methods are possibly easier and faster for differentiating reliably HCV types. These methods are based on antibody detection against serotype-specific epitopes of the hepatitis C virus [Tanaka et al., 1994]. They also allow detection of HCV RNA negative samples and can be used with specimens which have not received special handling. However, serological HCV typing may give false negative results because of a lack of sensitivity, and false results because of cross-reactivity between types [Simmonds et al., 1993c]. These drawbacks currently limit the use of serologic methods to establish the HCV types. It is thus of importance to establish the sensitivity and specificity of new serotyping methods by comparison with the reference method.

Recently, studies have compared results for the NS4 region [Simmonds et al., 1993c; Van Doorn et al., 1996; Bhattacherjee et al., 1995] and the core [Mondelli et al., 1994]. A few preliminary data were reported for the new RIBA HCV Serotyping SIA test [Dixit et al., 1995; Forns et al., 1996]. This test is an in vitro qualitative enzyme immunoassay and can discriminate between HCV infections with serotypes 1, 2, or 3. In the present study, the performance of this assay was assessed using a panel of 147 well-characterized French isolates from chronic hepatitis C patients.

The genotype determination of the different isolates was done by direct sequencing in the 5′ non-coding region. When this region did not suffice to establish reliably the genotype because its nucleotide sequence was not distinct enough, direct sequencing in the NS5-B region was undertaken.

PATIENTS AND METHODS

Patients

Sera were obtained from 147 anti-HCV positive (EIA III, Ortho Clinicals Diagnostics, France) French patients (87 males, 60 females) with histologically proven chronic hepatitis C infection. Informed consent to participate in the study was obtained from all patients. All specimens were well documented. They were collected and stored according to a protocol established to prevent contamination and degradation. All were stored at −80°C. All patients were positive by reverse-transcription PCR with primers from the 5′ non-coding region [Young et al., 1993]. These 147 sera were analyzed for genotyping by a serological and by a direct sequence analysis method.

Methods

RIBA HCV serotyping SIA assay. This assay uses an immunoblot EIA technique for the detection of antibodies to the serotype-specific epitopes of the hepatitis C virus. The assay utilizes 8 synthetic HCV encoded peptides. All of these are highly serotype-specific, with the exception of the serotype 1 core peptide which shows significant cross-reactivity with serotype 3 specimens [Mondelli et al., 1994]. Five peptides are from the serotype-specific sequences of the NS-4 non-structural coding region of the genome, and three peptides are from the core region of the different HCV isolates. The peptides are coated individually or in combinations of 2 peptides in each band of the serotyping strip. Band 1 contains the type 1a and 1b epitopes, band 2 contains the type 2a and 2b epitopes, and band 3 contains the type 3 epitope from the NS-4 region of HCV genome. Band 4 contains the type 1 epitopes, while band 5 contains type 2 epitope of the Japanese and Italian HCV variant from the core region. The assay was performed according to the manufacturer's instructions [Dixit et al., 1995]. The RIBA strip is interpreted for HCV serotype according to a previously described algorithm [Dixit et al., 1995]. When an equal signal was observed between two bands (NS4-1 and NS4-3), the test was interpreted as serotype 1 or 3 (not 1 *and* 3), following the recommendations of the manufacturer. The procedure lasts about 5 hours.

Direct sequence analysis. HCV RNA sequences from the 147 plasma samples were extracted, reverse transcribed, and amplified in the 5′NCR and NS-5 B region [Mellor et al., 1995; Simmonds et al., 1993a]. HCV RNA was extracted from serum through the guanidium salt, phenol, and chloroform method [Chomczynski and Sacchi, 1987] with RNAzol B (Biotecx Laboratories, Houston, Tex). Briefly, 25 microliters of serum was processed for RNA isolation. HCV RNA was detected by a reverse transcriptase-nested PCR [Young et al., 1993] with two sets of primers (5′NCR and NS-5 B region). The recommendations of Kwok and Higuchi [1989] were employed to avoid possible contamination.

The HCV type was determined by direct nucleotide sequencing of the PCR product with the Autoload DNA sequencing kit (Pharmacia Biotech, Uppsala, Sweden) and A.L.F. automatic DNA sequencer (Pharmacia Biotech). The degree of relatedness between HCV sequences was investigated by phylogenic analysis.

Statistical analysis. Percentages were compared by Fisher's exact test. Differences between proportions were analyzed through the chi-square test.

RESULTS

Genotyping by Direct Sequence Analysis

The distribution of the HCV genotypes identified by direct sequence analysis was as follows: the most common genotype was type 1 (59.2%), followed by type 3 (23.1%), type 2 (10.2%), type 4 (4.1%), and type 5 (3.4%). No mixed genotypes were found by this method.

TABLE I. Detection of Type-Specific Antibody in Serum Samples of HCV-Infected Patients of Known Genotype

	Number of samples with the following serotype									
Genotype[a]	1	2	3	1 or 2	1 or 3	NT	Total	Sensitivity (%)[b]	Specificity (%)[c]	Concordance (%)[d]
1 (all)	72	2	3	—	6	4	87	95.4	92.8	82.8
1a	29	1	1	—	2	0				
1b	43	1	1	—	4	4				
1c	—	—	1	—	—	0				
2(all)	1	8	2	1		3	15	80.0	91.7	53.3
2a	—	2	—	—		0				
2b	—	5	1	1	—	2				
2c	1	1	1	—	—	1				
3a	2	—	29	—	2	1	34	97.1	91.2	87.9
4(all)	3	—	2	—	—	1	6	83.3		
4a		—	1	—	—	1				
4b	3	—	1	—	—	0				
5a	1	1	—	—	1	2	5	60.0		
Total	79	11	36	1	9	11	147	92.5	92.6	85.7

[a]As determined by sequencing of 5′NC and NS5 regions, NT: no type-specific antibodies.
[b]Number of serotypeable samples/total number of samples.
[c]Number of correct serotypes/total number of serotypeable samples.
[d]Number of correct serotypes/total number of samples.

Genotyping by Serological Assay

A total of 147 serum samples from chronically infected patients were analyzed by the RIBA HCV serotype. Type specific antibodies were detected in 136 of the 147 isolates (92.5%). Overall, 126 of 136 (92.6% specificity) serotypable samples (85.7% of the total 147) revealed a serotype concordant with genotype (concordance was defined as the percentage of correct serotypes/total number of samples). Of the 126 HCV serotyped specimens, 62.7% were type 1, 8.7% were type 2, and 28.6% were type 3. Serotype 1 or 2 was found in one patient, and serotype 1 or 3 was found in nine patients.

Comparison of Methods

The serotypes of 147 specimens were compared with the genotype findings by sequence analysis (Table I). Of the 136 serotypable samples, 18 (13.2%) revealed a serotype different from the corresponding genotype. These included five samples for genotype 1, three for genotype 2, two for genotype 3, five for genotype 4, and three for genotype 5. Repetition of serotyping for these samples yielded identical results. The intensities of the bands for these discrepant samples did not differ from those for concordant specimens.

The isolate names and RIBA SIA patterns with discrepant results are shown in Table II. In these cases no particular or novel isolates were associated with a particular pattern. Ten patients showed a reactivity with mixed serotypes; one had circulating antibodies to type 1 or 2 and nine had circulating antibodies to type 1 or 3. Nine samples were typed as either 1 or 3 because results with core were higher than NS4 peptide bands or NS4-1 and NS4-3 bands were equal. Except for genotypes 4 and 5, the two assays were discordant for only 10 patients (6.8%). The overall sensitivity and specificity of the RIBA SIA HCV serotyping assay were 92.5% and 92.6%, respectively (the sensitivity of the assay was defined as the number of serotypable samples/total number of samples; the specificity of the assay was defined as number of correct serotypes/total number of serotypable samples). Note that among the 11 nonserotypable samples, many samples that reacted with 4^+ intensity to the RIBA-3 c 22 peptide were nonreactive to the core peptide in serotype SIA.

DISCUSSION

Only sequence analysis of specific gene regions of the HCV genome that are predictive of genotype is completely reliable for genotyping [Bukh et al., 1995; Simmonds et al., 1995]. However, this method, based on PCR is technically complex and time consuming. Moreover, it cannot be undertaken in cases with low-level viremia or if serum samples have not been stored correctly [Halfon et al., 1996].

To overcome these drawbacks, many have sought to develop simple serological techniques to identify the main types and subtypes. These serological typing methods have advantages in terms of speed, simplicity, and cost. These convenient methods may theoretically replace the PCR-based methods. Their accuracy is debated [Bhattacherjee et al., 1995; Cerino et al., 1996]. The differences observed are related to (1) the HCV population studied: chronic HCV infected patients, IVDUs, hemodialysis patients, hemophiliacs, and blood donors [Jarvis et al., 1994; Van Doorn et al., 1996] and (2) the source of epitopes used in the test (core/NS4). Thus, a comparative analysis of the specificity and sensitivity of these methods may provide useful information. In the present study, we assessed the performance of a serotyping method based on core and NS4 antibody detection by comparison with direct sequencing in 5′ NCR and NS-5B in 147 French patients with chronic hepatitis C virus infection. The overall distribution of

TABLE II. Genotyped HCV Isolates That Give Discordant Serotypic Results

	RIBA SIA HCV serotyping strip band pattern					
	NS4			Core		
Isolates	1	2	3	1	2	Serotype
1a-HC1-N4	—	2^+	4^+	4^+	1^+	3
1a-US10/L38352	—	2^+	3^+	3^+	4^+	2
1b-HCVBK/D31602	—	—	—	—	3^+	2
1b-HCVBK/D31602	—		4^+	3^+	2^+	3
1c-HC-G9	—	—	1^+	1^+	1^+	3
2a/2c-9 access	—	1^+	3^+	—	—	3
2c-BE 134/L38320	3^+	—	—	—	—	1
2c-BE 134/L38320	—	—	1^+	—	—	3
3a-NZL1/L29466	3^+	—	2^+	4^+	4^+	1
3a-NZL1 ou BR 56	4^+	—	3^+	—	—	1
4a/4c-U33432	—	—	1^+	4^+	4^+	3
4a/4c-U33432	4^+	—	—	3^+	3^+	1
4a/4c-U33432	3^+	—	2^+	3^+	2^+	1
4a/4c-U33432	1^+	—	2^+	—	—	3
4a/4c-HC4-N5	3^+	—	—	1^+	—	1
5a-QC21	—	—	—	3^+	4^+	2
5a-SA	4^+	—	4^+	—	—	1 or 3
5a-SA	3^+	—	1^+	3^+	4^+	1

genotypes in these patients was in agreement with another French study [Martinot-Peignoux et al., 1995].

The sensitivity of the RIBA SIA HCV, serotyping assay (92.5%) was good and comparable with those of other studies [Bhattacherjee et al., 1995; Forns et al., 1996]. However, only 126 (including the mixed results) of 136 [92.6%] serotypable samples (85.7% of the total 147) revealed a serotype which was concordant with genotype.

Nine samples were found with serotype 1 or 3 and one with serotype 1 or 2; these results are probably in relation with a cross-reactivity of core peptide 1 with the HCV 3 genotype. Effectively, recent data [Forns et al., 1996] on mixed infections in 5′NC investigated by cloning and sequencing several clones for the same patient confirmed the low frequency of mixed infection due to nonspecific priming in this region. Note that if these 10 samples with mixed results were considered as non-concordant, the sensitivity of the assay would be 126/147 (85.7%) and the specificity 109/136 (80.1%).

The untypable samples (7.5%) may be due to the absence of antibodies towards the serotype peptides. Indeed, it was suggested that humoral response against some antigenic regions of HCV may be weak or absent in some patients [Mondelli et al., 1994]. The good sensitivity of the RIBA HCV 3.0 SIA assay (92.5%) is linked with the high level of circulating antibodies expected in chronically infected HCV patients.

It should be pointed out that the concordance observed between serotyping and definitive genotyping results was lower than that found by Dixit et al., (99%) using the same test in a similar population [Dixit et al., 1995]. The antigenic similarity of HCV (particularly between 1a and 1b) constitutes an obstacle for the development of a serotyping method to detect infection with different subtypes. For epidemiological studies, it is crucial to have of the informations on subtypes. For clinical assessment, it is not yet established that one must differentiate between subtypes. Genotype 1 (including subtypes 1a and 1b) has been shown to be associated with a poor response to interferon alpha therapy, whereas genotypes 2 and 3 would be better responders [Martinot-Peignoux et al., 1995]. Moreover, a clear relationship between HCV serotypes and response to interferon alpha treatment has been demonstrated [Jarvis et al., 1996; Chemello et al., 1994].

In summary, except for genotypes 4 and 5, the results of the RIBA HCV 3.0 SIA serotype test showed a good correlation (85.7%) with the direct sequence genotyping. The serotyping test is rapid and does not require the strict conditions of HCV RNA storage and preservation needed for direct sequence genotyping. However, although it is convenient, the HCV serotyping assay has a lower sensitivity than the molecular typing method. Also, the discrepant results limit its routine clinical use. We recommend that further peptides for HCV 1–3 be incorporated in this assay to improve its sensitivity and specificity for these types. Moreover, the addition of peptides of HCV 4–6 and the definition of HCV subtypes would be appreciated for use in the clinical context. This may be resolved by using more variable regions of the genome.

REFERENCES

Bhattacherjee V, Prescott LE, Pike I, Rodgers B, Bell H, Elzaadi AR, Kew MCV, Conradie J, Lin CK, Marsden H, Saeed AA, Parker D, Yap PL, Simmonds P (1995): Use of the NS-4 peptides to identify type-specific antibody to hepatitis C virus genotypes 1, 2, 3, 4, 5 and 6. Journal of General Virology 76:1737–1748.

Bukh J, Purcell RH, Miller RH (1993): At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected in the worldwide. Proceedings of the National Academy of Sciences USA 90:8234–8238.

Bukh J, Purcell RH, Miller RH (1994); Sequence analysis of the core gene of 14 hepatitis C genotypes. Proceedings of the National Academy of Sciences USA 91:8239–8243.

Bukh J, Miller RH, Purcell RH (1995): Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. Seminar of Liver Diseases 15:41–63.

Cerino A, Cividini A, Asti M, Lanza A, Silini, Mondelli M (1996): Comparative evaluation of two serologic typing methods for hepatitis C virus. Journal of Clinical Microbioliology 34:714–716

Chayama K, Tsubota A, Arase Y, Saitoh S, Koida I, Ikeda K, Matsumoto T, Kobayashi M, Iwasaki S, Koyama S, Morinaga T, Kumada H (1993): Genotypic subtyping of hepatitis C virus. Journal of Gastroenterological Hepatology 8:150–156.

Chemello L, Alberti A, Rose K, Simmonds P (1994): Hepatitis C serotype and Interferon response to interferon therapy. New England Journal of Medicine 330:143.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162:156–159.

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M (1989): Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359–362.

Dixit V, Quan S, Martin P, Larson D, Brezina M, Dinello R, Sra K, Lau JYN, Chien D, Kolberg J, Tagger A, Davis G, Polito A, Gitnick G (1995): Evaluation of a novel serotyping system for hepatitis C virus: strong correlation with standard genotyping methodologies. Journal of Clinical Microbiology 33:2978–2983.

Forns X, Maluenda MD, Lopez-Labrador FX, Ampurdanes S, Olmedo E, Costa J, Simmonds P, Sanchez-Tapias S, Jimenez De Anta MT, Rodes J (1996): Comparative study of three methods for genotyping hepatitis C virus strains in samples from Spanish patients. Journal of Clinical Microbiology 34:2516–2521.

Halfon P, Khiri H, Gerolami V, Feryn JM, Reynier P, Bourliere M, Cartouzou G (1996): Impact of various handling and storage conditions on quantitative detection of hepatitis C virus RNA. Journal of Hepatology 25:307–311.

Holmes EC, Simmonds P, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS (1993): Derivation of a rational nomenclature for hepatitis C virus by phylogenetic analysis of the NS-5 region. In Nishioka K, Suzuki H, Mishiro S, and Oda T (eds): "Viral Hepatitis and Liver Disease." Tokyo: Springer-Verlag, pp 57–62.

Jarvis LM, Watson HG, McOmish F, Peutherer JF, Ludlam CA, Simmonds P (1994): HCV genotype variation in haemophiliacs. Journal of Infectious Diseases 170:1018–1022.

Kwok S, Higuchi R (1989): Avoiding false positives with PCR. Nature 339:237–238.

Martinot-Peignoux M, Marcellin P, Pouteau M, Castelnau C, Boyer N, Poliquin M, Degott C, Descombes I, Le Breton V, Milotova V, Benhamou JP, Erlinger S (1995): Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factor of sustained response to interferon alfa therapy in chronic Hepatitis C. Hepatology 22:1050–1056.

Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P and the International HCV Collaborative Study Group (1995): Investigation of pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. Journal of General Virology 16:2493–2507.

Mondelli MU, Cerino A, Bono F, Cividini A, Maccabruni A, Arico M, Malfitano A, Barbarini G, Piazza V, Minoli L, Silini E (1994): Hepatitic C virus (HCV) core serotypes in chronic HCV infection. Journal of Clinical Microbiology 32:2523–2527.

Nousbaum JB, Pol S, Nalpas B, Landais P, Berthelot P, Brechot C and a Collaborative Study Group (1995): Hepatitis C virus type 1b (II) infection in France and Italy. Annals Internal Medicine 122:161–168.

Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M (1992): Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. Journal of General Virology 73:673–679.

Pawlotsky JM, Tazkiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, Dhumeaux D (1995): Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. Journal of Infectious Diseases 171:1607–1610

Simmonds P (1995): Variability of hepatitis C virus. Hepatology 21: 570–581

Simmonds P, Homes EC, Cha T-A, Chan SW, McOmishi F, Irvine B, Beall E, Yap P, Kolberg J, Urdea MS (1993a): Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. Journal of General Virology 74:2391–2399.

Simmonds P, McOmish F, Yap PL, Chan SW, Lin CK, Dusheiko G, Saeed AA, Holmes EC (1993b): Sequence variability in the 5′ noncoding region of hepatitis C virus. Identification of a new virus type and restrictions on sequence diversity. Journal of General Virology 74:661–668.

Simmonds P, Rose KA, Graham S, Chan SW, McOmish F, Dow BC, Follett EAC, Yap PL, Holmes EC (1993c): Mapping of serotype-specific, immunodominant epitopes in the NS4-region of hepatitis C virus (HCV)—use of type-specific peptides to serologically differentiate infections with HCV type 1, type 2, and type 3. Journal of Clinical Microbiology 31:1493–1503.

Stuyver L, Rossau R, Wyseur A, Duhamel M, Vanderborght B, Van Heuverswyn H, Maertens G (1993): Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. Journal of General Virology 74:1093–1102.

Tanaka T, Tsukiyama Kohara K, Yamaguchi K, Yagi S, Tanaka S, Hasegawa A, Ohta Y, Hattori N, Kohara M (1994): Significance of specific antibody assay for genotyping of hepatitis C virus. Hepatology 19:1347–1353.

Tokita H, Shrestha SM, Okamoto H, Sakamoto M, Horikita M, Lizuka H, Shrestha S, Miyakawa Y, Mayumi M (1994): Hepatitis C virus variants from Nepal with novel genotypes and their classification into the third major group. Journal of General Virology 75:931–936.

Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromistsu K 91994): Factor predictive of response to interferon-a therapy in hepatitis C virus infection. Hepatology 19:1088–1094.

Van Doorn L-J, Kleter B, Pike I, Quint W (1996): Analysis of hepatitis C virus isolates by serotyping and genotyping. Journal of Clinical Microbiology 34:1784–1787.

Young KKY, Resnick RM, Myers TW (1993): Detection of hepatitis C virus RNA by a combined reverse transcription-polymerase chain reaction assay. Journal of Clinical Microbiology 31:882–886.